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Copyright © 1987 by The Endocrine SocietyTHE SYRIAN HAMSTER PINEAL GLAND RESPONDS TO ISOPROTERENOL IN VIVO AT NIGHTGeorge M. Vaughan¹ and Russel J. Reiter²¹US Army Institute of Surgical Research, Ft. Sam Houston, Texas 78234-6200 and
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ABSTRACT: Failure of isoproterenol (ISO) injections to raise pineal melatonin content has generated doubt about β -adrenergic control of the melatonin rhythm in Syrian hamsters. However, the effect of ISO injected at night after light-induced reduction of pineal melatonin has not been reported. In this study, light exposure began at 6 1/4 h into one (normally 10-h) dark phase. The hamsters were injected with either ISO (1 mg/kg) or vehicle 15 min later when pineal melatonin content was low. Light exposure continued. Two h after ISO but not vehicle injection, pineal melatonin content rose more than six-fold. In other animals injected at the end of the usual light phase then kept in light for 2 h, pineal melatonin was equally low after ISO or vehicle injection. The Syrian hamster pineal gland can respond *in vivo* to a β -adrenergic agonist injected at the physiologically relevant time of the normal nocturnal melatonin surge. This finding, taken together with the previously reported inhibition of the endogenous nocturnal melatonin surge with a β -blocking drug, suggests that a β -adrenergic mechanism controls the hamster pineal melatonin rhythm.

In rats, β -adrenergic agonists injected during daytime stimulate pineal melatonin synthesis (1-5). In contrast, Syrian hamster pineal melatonin does not rise in response to isoproterenol (ISO) in either the light (6-8) or dark (9) phase of the normal cycle. The purpose of this study was to determine if hamster pineal melatonin content rises after injection of ISO when the nocturnal melatonin surge is acutely blocked by light.

MATERIALS AND METHODS

Adult male Syrian hamsters (*Mesocricetus auratus*) were adapted for at least one month to a 14L:10D light cycle (lights off: 2000-0600 h). They were given a standard rodent chow and water *ad libitum*. Light ("cool white" fluorescent) intensity was 75 foot-candles at the front of the cages. Each experiment was done on a different night. The animals were killed by decapitation, and the pineal was removed, frozen on dry ice and sonicated in assay buffer. Homogenates were stored at -60°C until RIA of melatonin (10). Sampling in the dark was carried out under dim red light (two 25-w incandescent globes, each shielded with a Kodak No. 1A filter). Results were analyzed by *t* tests with the Bonferroni correction for multiplicity of comparisons.

In experiment (exp.) 1, 0.1 ml of either vehicle or ISO (1 mg/kg) was injected *sc* at the end of a light phase. Animals (8/group) were kept in light for 2 h until sampling.

In exp. 2, 5 hamsters were sampled in the dark at 0215 h. At this time, 31 other hamsters were transferred into a lighted room for subsequent sampling in order to determine when pineal melatonin content reached daytime levels. Animals were grouped for the time intervals after beginning the light exposure: 2 animals at 0-5 min, 3 at 5-10 min, 4 at 10-15 min,

3 at 15-20 min, 7 at 20-30 min, 9 at 30-40 min, and 3 at 40-50 min. A best-fit decay line was obtained by a single 3-parameter exponential regression of the individual data.

In exp. 3 (7-8/group), 2 groups of hamsters were sampled in the dark; one at 0215 h and the other 2 h later. Three other groups were brought into a lighted room at 0215 h; 1 was sampled 15 min later at 0230 h, and the other 2 were injected *sc* at 0230 h with 0.1 ml of either 0.15 M NaCl vehicle or ISO (1 mg/kg) and kept in light for 2 h until sampling. In exp. 4, groups of 7-8 hamsters were sampled with the same protocol as in exp. 3, except there was no group sampled at 0215 h in the dark.

RESULTS

ISO and vehicle injections at the end of the light phase resulted in similar pineal melatonin content (exp. 1, Fig. 1A), characteristic of normal daytime values in this laboratory (10, 11).

During the second half of the dark phase (Fig. 1B), by 15 min in novel light exposure, pineal melatonin content in uninjected animals (exp. 2) was typical for the end of the light phase (11). Hamsters sampled 2 h after receiving saline had pineal melatonin contents not different from values expected at the time of injection. 2 hours after injection of ISO, the values were significantly higher ($P < 0.001$) than those obtained either after saline or at the time of injection (combined exp. 3 and 4). The values for hamsters killed in the dark were typical of those found during the normal nocturnal surge of pineal melatonin synthesis (10, 11).

DISCUSSION

In a previous study of Syrian hamsters (7), injection of 3 or 10 mg/kg ISO *sc* during the day (4-9 h into the light phase) produced no response in pineal melatonin content assessed at intervals from 40 min to

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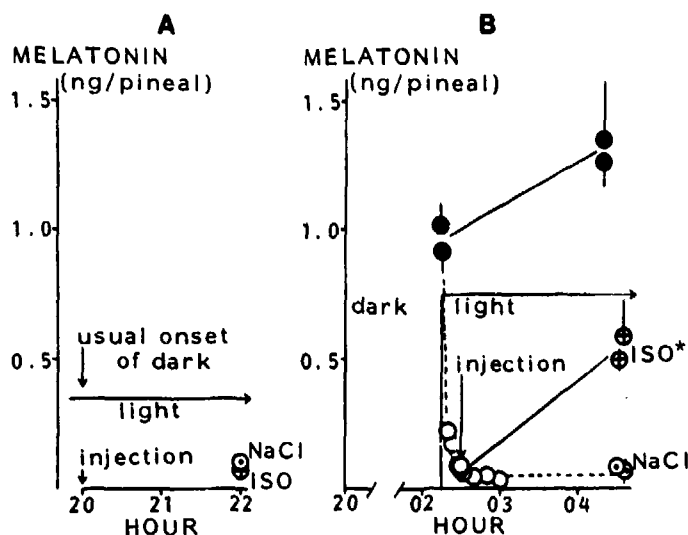


Fig. 1. Syrian hamster pineal melatonin content (mean \pm SE): A (exp. 1), after isoproterenol (ISO, 1 mg/kg) or saline vehicle (NaCl) injected at the end of the usual light phase; B (exp. 2-4), after injection of ISO (crossed circles) or NaCl (dotted circles) or no injection (open circles) during exposure to light from 0215 h, or after no injection and no nocturnal light exposure (closed circles). The dashed line represents the best-fit curve for the data of exp. 2 with a decay constant of $-0.31/\text{min}$ and half-time of $2\frac{1}{4}$ min. * $p < 0.001$, combined ISO groups vs. those having received NaCl or those sampled at 0230 h without injection.

5 h after injection. When 3 or 6 mg/kg of ISO was injected at the end of the light phase or in the dark phase, there was no detectable advancement or enhancement of the nocturnal melatonin surge (9). Such results seem incongruent with the previously shown ability of pharmacologic β -blockade to prevent the normal *in vivo* nocturnal surge of pineal melatonin content in this species (12). However, norepinephrine (NE) released from the intrinsic nerve endings probably is highly concentrated at the pinealocyte during the nocturnal surge of pineal sympathetic activity (11, 13) and may allow little additional influence of injected ISO. We were interested in whether ISO, injected at night, could raise pineal melatonin from light-induced low levels before insensitivity might develop. Previous *in vitro* studies (14) had indicated that light exposure of hamsters for 6 h at night inhibited a response of pineal melatonin synthesis during incubation with NE.

After blocking neuronal uptake with desipramine, we previously stimulated a rise in hamster pineal melatonin by injecting NE 30 min following onset of lighting at night (14; Reiter, Vaughan, Oaknin, unpublished results). In the present study, we used ISO because it is not inactivated by neuronal uptake and does not require use of another drug for blockade of uptake in pineal nerve endings (15), and the α activity of NE might influence pineal melatonin synthesis (16). Thus, we injected ISO 2 h before the

normal time of the peak melatonin content and after the shortest light exposure able to depress melatonin to daytime levels. Injected at 0230 h after 15 min light, ISO produced a 6.7-fold rise in pineal melatonin content. Since no effect was seen after ISO given at 2000 h, it appears that sensitivity to ISO develops some time during the dark phase. The insensitivity of the hamster pineal at the end of the light phase appears impressive. Pineals taken then and incubated with doses of ISO up to $10\text{ }\mu\text{M}$ had no response of melatonin synthesis, yet those taken after brief light exposure 6 h into the dark phase had a dramatic response beginning with ISO concentration at least two logs lower (17). The present results show that a difference between these two time points can be demonstrated *in vivo*.

In a previous report, ISO (10 mg/kg ip) injected in the dark, when pineal melatonin was high, inhibited a fall in hamster pineal melatonin content after subsequent light exposure (9). Though this is in agreement with the present finding of nocturnal responsiveness to ISO, those studies did not include injection of ISO at night shortly after light exposure, and thus did not demonstrate the ability of ISO to stimulate pineal melatonin from low levels. The present results demonstrate this and help substantiate the view that in Syrian hamsters, as in rats, the pineal melatonin rhythm is controlled by a β -adrenergic mechanism.

These findings differ markedly from the reported peak responsiveness of the pineal at the end of the light phase when there has been the least endogenous stimulation of the pineal and the least down-regulation of catecholamine receptors on pinealocytes (3-5, 18). These reports of rats with denervated pineals or of intact rats during a normal or prolonged light phase did not assess responsiveness during the time of the nocturnal melatonin surge after exposure to darkness for part of the night. In fact, *in vitro* results indicate that even in the rat, which maintains easily demonstrable pineal β -responsiveness during the day, pineal glands taken 6 1/2 h into the night after a period of darkness are more responsive than those taken at the end of the day (17). Our results in the hamster substantiate this view. Whether the nocturnal responsiveness in hamster pineal glands results from a change at the receptor or post-receptor level is unknown, though catecholamine receptor density apparently increases at night in rat pineal (19).

The elevated pineal melatonin content after nocturnal ISO injection did not reach the level seen during the endogenous nocturnal surge. This might be due in part to a sub-optimal dose of ISO and/or time of injection or sampling. Dose-response trials for different times of ISO injection or of sampling have not been performed, though experiments with NE injection after brief light exposure in desipramine-primed hamsters indicate that the period of maximal sensitivity may be the second half of the dark phase (Reiter, Vaughan and Oaknin,

unpublished results. Another factor, already suggested (17), may be that the brief light exposure itself before and during the action of the injected agonist might have partially induced insensitivity of the pineal. Besides the nocturnal stimulatory effect of ISO on hamster pineal melatonin synthesis (17), ISO may also minimally enhance elimination of melatonin from the pineal at night as suggested indirectly by *in vitro* results (17). Whether such an effect might have dampened the rise in melatonin content after *in vivo* ISO injection at night is not known.

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REFERENCES

1. Deguchi T, Axelrod J 1972 Control of circadian change of serotonin N-acetyltransferase activity in the pineal organ by the beta-adrenergic receptor. *Proc Nat Acad Sci USA* 69:2547
2. Deguchi T, Axelrod J 1973 Superinduction of serotonin N-acetyltransferase and supersensitivity of adenyl cyclase to catecholamines in denervated pineal gland. *Mol Pharmacol* 9:612
3. Deguchi T, Axelrod J 1974 Supersensitivity and subsensitivity of the beta-adrenergic receptor in pineal gland regulated by catecholamine transmitter. *Proc Nat Acad Sci USA* 70:2411
4. Brownstein M, Saavedra JM, Axelrod J 1973 Control of pineal N-acetylserotonin by a beta adrenergic receptor. *Mol Pharmacol* 9:605
5. Romero JA 1976 Influence of diurnal cycles on biochemical parameters of drug sensitivity: the pineal gland as a model. *Fed Proc* 35:1157
6. Binkley S 1976 Comparative biochemistry of the pineal gland of birds and mammals. *Am Zool* 16:57
7. Lipton JS, Petterborg LJ, Steinlechner S, Reiter RJ 1982 *In vivo* responses of the pineal gland of the Syrian hamster to isoproterenol or norepinephrine. In: Reiter RJ (ed) *The Pineal and its Hormones*. Alan R Liss Inc, New York, p 107
8. Steinlechner S, King TS, Champney TH, Spaniel-Borowski K, Reiter RJ 1984 Comparison of the effects of beta-adrenergic agents on pineal serotonin N-acetyltransferase activity and melatonin content in two species of hamsters. *J Pineal Res* 1:23
9. Steinlechner S, King TS, Champney TH, Richardson BA, Reiter RJ 1985 Pharmacological studies on the regulation of N-acetyltransferase activity and melatonin content of the pineal gland of the Syrian hamster. *J Pineal Res* 2:109
10. Vaughan GM, Taylor TJ, Pruitt BA Jr, Mason AD Jr 1985 Pineal function in burns: Melatonin is not a marker for general sympathetic activity. *J Pineal Res* 2:1
11. Vaughan GM, Reiter RJ 1986 Pineal dependence of the Syrian hamster's nocturnal serum melatonin surge. *J Pineal Res* 3:9
12. Lipton JS, Petterborg LJ, Reiter RJ 1981 Influence of propranolol, phenoxybenzamine or phentolamine on the *in vivo* nocturnal rise of pineal melatonin levels in the Syrian hamster. *Life Sci* 28:2377
13. Craft CM, Morgan WW, Reiter RJ 1984 24 hour changes in catecholamine synthesis in rat and hamster pineal glands. *Neuroendocrinology* 38:193
14. Vaughan GM, Lasko J, Coggins SH, Pruitt BA Jr, Mason AD Jr 1986 Rhythmic melatonin response of the Syrian hamster pineal gland to norepinephrine *in vitro* and *in vivo*. *J Pineal Res* 3:235
15. Parfitt AG, Klein DC 1976 Sympathetic nerve endings in the pineal gland protect against acute stress-induced increase in N-acetyltransferase (EC 2.3.1.5.) activity. *Endocrinology* 99:840
16. Vanecek J, Sugden D, Weller J, Klein DC 1985 Atypical synergistic alpha₁- and beta-adrenergic regulation of adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate in rat pinealocytes. *Endocrinology* 116:2167
17. Vaughan GM, Pruitt BA Jr, Mason AD Jr 1986 Nyctohemeral rhythm in melatonin response to isoproterenol *in vitro*: Comparison of rats and Syrian hamsters. *Comp Biochem Physiol*, 1986 (in press).
18. Romero JA, Axelrod J 1974 Pineal beta-adrenergic receptor: Diurnal variation in sensitivity. *Science* 184:1091
19. Reiter RJ, Esquifino AI, Champney TH, Craft CM, Vaughan MK 1985 Pineal melatonin production in relation to sexual development in the male rat. In: Gupta D (ed), *Paediatric Endocrinology*. Croon Helm, London, p: 190



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